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Recommendations for pen-based oral-fluid collection in growing pigs

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Abstract
Sampling guidelines were developed by observing pigs during oral-fluid sample collection in commercial herds. Pigs with previous oral-fluid collection experience ("trained") should be allowed 20 minutes access to the rope. Pigs with no prior experience ("untrained") should be allowed 60 minutes. One collection is enough to train pigs.

Keywords
swine, pig behavior, oral fluid, sampling, surveillance

Disciplines
Large or Food Animal and Equine Medicine | Other Veterinary Medicine | Statistical Methodology

Comments
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Brief communication

Peer reviewed

Recommendations for pen-based oral-fluid collection in growing pigs

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Summary

Sampling guidelines were developed by observing pigs during oral-fluid sample collection in commercial herds. Pigs with previous oral-fluid collection experience ("trained") should be allowed 20 minutes access to the rope. Pigs with no prior experience ("untrained") should be allowed 60 minutes. One collection is enough to train pigs.

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Resumen - Recomendaciones para la recolección de fluidos orales en corrales de cerdos en crecimiento

Se desarrolló una guía de muestreo al observar a cerdos durante la recolección de muestras de fluidos orales en hatos comerciales. A los cerdos con experiencia previa ("entrenados") en la recolección de fluidos orales se les debe permitir 20 minutos de acceso a la cuerda. A los cerdos sin experiencia previa ("no entrenados") se les debe permitir 60 minutos. Una recolección es suficiente para entrenar a los cerdos.

Résumé - Recommandations pour le prélèvement dans les enclos de fluides oraux provenant de porcs en croissance

Des directives pour l'échantillonnage furent développées suite à l'observation de porcs durant le prélèvement d'échantillons de fluides oraux dans des troupeaux commerciaux. Pour des porcs ayant déjà vécu l'expérience de prélèvement de fluides oraux ("entrainés") ont devrait allouer un accès de 20 minutes à la corde. Pour des porcs sans expérience antérieure ("non-entrainé") ont devrait allouer 60 minutes. Une session de prélèvement est suffisante pour entrainer des porcs.

Although oral-fluid-based testing was introduced to swine medicine relatively recently, it has been widely accepted by the pork industry. In 2010, the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) performed 10,329 tests on porcine oral-fluid samples. This number increased to 32,544 in 2011, 60,172 in 2012, and 94,011 in 2013 (written communication, Dr Rodger Main, 2014). Although currently an area of development and research, assays described for oral-fluid specimens include antibody- and PCR-based assays for a variety of pathogens, eg, porcine reproductive and respiratory syndrome virus,[1,2] influenza A virus,[3] porcine circovirus type 2,[4] African swine fever virus,[5] and others. In the field, detection of an analyte in a pen-based oral-fluid specimen depends on the prevalence of the infection in the pen,[6] stage of the infection and kinetics of the immune response,[1-4,7] the diagnostic performance of the PCR-based or antibody-based assay used to test the sample,[6,8,9] and pig behavior associated with sample collection, eg, the number of pigs that contribute oral fluid to the sample. Although the published research has begun to address many of these issues, research on pig behavior relevant to oral-fluid sampling is scarce. The primary objective of this study was to answer the question "How long should a sampling rope be left in place to achieve the best representation of the pigs in the pen?"

Materials and methods

All animal handling, housing, and veterinary care was approved and supervised by Murphy Brown LLC and conformed to Pork Quality Assurance Plus guidelines (**www.pork.org**). In addition, the Iowa State University Institutional Animal Care and Use Committee reviewed and approved the research study.

Animals, housing, and oral-fluid collection

Observations were made in five commercial barns located on four sites. All barns were naturally ventilated and each housed approximately 1100 animals in 40 pens, ie, 25 to 28 animals per pen. All pens were equipped with nipple waterers, and adjoining pens shared feeders. Pens in barns 1 and 4 were equipped with completely slatted floors and metal gates, whereas pens in barns 2, 3, and 5 had partially slatted floors and concrete pen dividers.

To collect oral fluids, cotton rope (0.5-inch three-strand twisted 100% cotton rope; Web Ringing Supply, Lake Barrington, Illinois) was suspended in each pen for 30 minutes. In barns 1 and 4, rope was suspended from the arm of a bracket (2-inch boxed steel) bolted to the bars of the metal gates. In barns 2, 3, and 5, the rope was suspended from a short chain attached to a bracket hooked to the rafters. In all cases, the rope was placed at least 0.6 m from the sides of the pen and suspended with the end of the rope at the pigs' shoulder level. In this arrangement, the rope could be placed and recovered

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from the aisle without entering the pen or disturbing the pigs.

Oral-fluid samples were collected from barns 1, 2, 3, and 4 each day for 5 days prior to behavioral observations (training period), whereas no oral-fluid samples were collected from Barn 5 prior to behavioral observations. Hereafter, the pigs in barns 1 to 4 will be referred to as "trained" and pigs in Barn 5 as "untrained."

Collection of behavioral data related to oral-fluid sampling

Behavior data were collected by direct observation of 233 six- to 12-week-old pigs housed in 163 pens with approximately 4100 pen mates. Observed pigs consisted of convenience-sampled, age-matched pigs from within the system. These pigs were clearly marked on the dorsal aspect of the body from the neck to the rump (Prima Spray On; Prima Tech, Kenansville, North Carolina) to differentiate them from pen mates. One trained pig (barns 1, 2, 3, 4) was added to each pen (n = 143) of 25 to 28 pigs and then observed the following day during one 30-minute collection. Observations on untrained pigs (Barn 5) were made on variable numbers of marked pigs in pens (n = 20) holding 25 pigs: one pig was observed in each of five pens, three pigs in five pens, five pigs in five pens, and nine pigs in five pens for a total of 90 pigs. In untrained pigs, observational data were collected for five successive 30-minute collections to allow for documentation of learning behavior.

Behavioral data were collected by pen-side observers, each equipped with a timer and data recording sheet during 30-minute sampling periods. To quantify pig interactions with the rope, the observation period was divided into 30 one-minute intervals, and each minute was classified as "yes" or "no" for positive contact. "A positive contact" was defined as observing the marked pig take the rope into its mouth, regardless of the length of time the rope was in the pig's mouth. The trained pigs in barns 1, 2, 3, and 4 were observed for one observation period, whereas the untrained pigs in barn 5 were observed for five consecutive 30-minute observation periods. Thereafter, the data were used to describe the observed pig behaviors, eg, the percent of pigs that contacted the rope over the 30-minute observation period, cumulative rope contacts, and other behavioral patterns related to interacting with the rope.

Statistical analysis comparing behavioral data for barns 1 through 5 was performed using an ANOVA in SAS version 9.2 (SAS Institute, Inc, Cary, North Carolina).

Behavioral outcomes included cumulative interaction with the rope over time, pig total contact time, and patterns of pig contact over time. Descriptive statistics of other data are presented.

Results

No significant differences in pig behavior were noted between barns 1, 2, 3, and 4 (trained pigs), but observations in Barn 5 (untrained pigs) differed significantly (ANOVA; $P < .01$) from those in the other barns. On the basis of this analysis, the data from barns 1 through 4 were combined for subsequent analyses.

Among trained pigs, 16.1% to 31.4% (mean 22.9%) of the observed pigs (n = 143) contacted the rope during each 1-minute interval of the observation period (Figure 1). Cumulatively, 86 (60.1%) of the referents contacted the rope in the first 10 minutes of the observation period, 101 (70.6%) in the first 20 minutes, and 108 (75.5%) in 30 minutes; 35 (24.4%) never contacted the rope (Figure 1). Among the pigs that interacted with the rope, 49 (34.2%) interacted for ≤ 5 minutes, 21 (14.6%) for 6 to 10 minutes, 20 (13.8%) for 11 to 15 minutes, 13 (9.1%) for 16 to 21 minutes, and 5 (3.5%) for 21 minutes or more (Figure 2). The mean total contact time among the 108 pigs contacting the rope was 6.9 minutes. Twenty-two (15.4%) of the pigs that contacted the rope did so in a single contact event, but most cycled away from and back to the rope (Figure 3). Thus, two contact events were observed in 36 (25.2%) of the pigs, three events in 20 (14.0%), four events in 18 (12.6%), and ≥ 5 in 12 (8.4%).

Untrained pigs (n = 90) were monitored in five sequential 30-minute observation periods. In the first observation period (Observation 1), 6.7% to 28.8% of the pigs (mean 21.1%) contacted the rope during each 1-minute interval (Figure 4). Cumulatively, 30 (33.3%) contacted the rope in the first 10 minutes, 40 (44.4%) in the first 20 minutes, and 49 (54.4%) in 30 minutes; 41 (45.6%) never contacted the rope (Figure 4). Pig adaptive behavior was apparent in observations 2 through 5 (Figure 5). These observations differed from Observation 1, but not from each other. Thus, in observations 2 through 5, a mean of 48 (53.3%)

Figure 1: Percent of "trained pigs" (n = 143) interacting with an oral-fluid collection rope over the observation period. A total of 233 six- to 12-week-old pigs in a commercial finisher were observed. Trained pigs were defined as having previous experience with oral-fluid collection. Behavioral data were collected by pen-side observers each equipped with a timer and data recording sheet during 30-minute sampling periods. To quantify pig interactions with the rope, the observation period was divided into 30 one-minute intervals, with each minute classified as "yes" or "no" for positive contact. A "positive contact" was defined as observing the pig taking the rope into its mouth, regardless of the length of time the rope was in the pig's mouth.

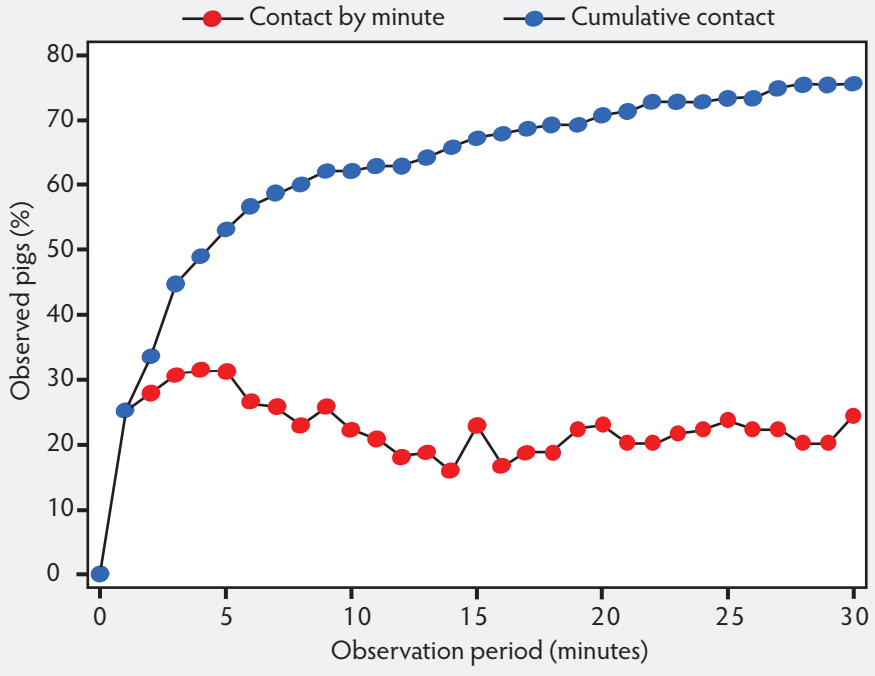


Figure 2: Distribution of total contact time with the rope among trained pigs as described in Figure 1 (n = 143).

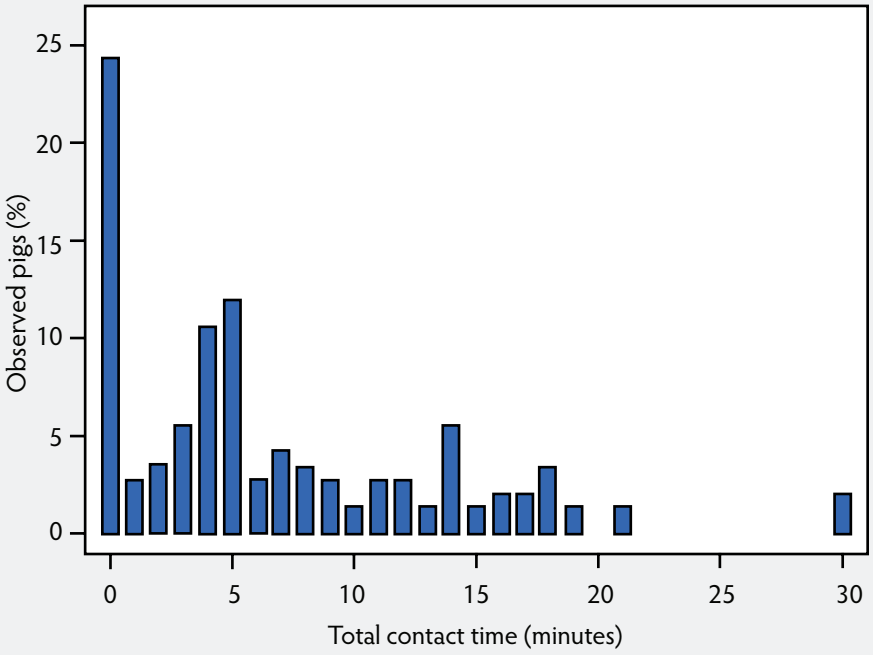
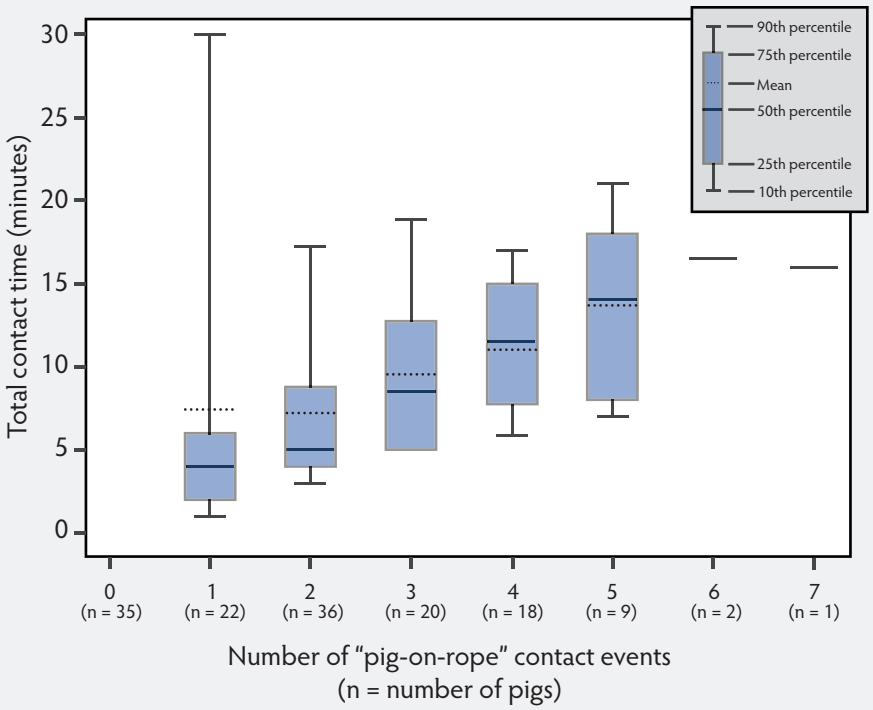


Figure 3: Number of positive-contact events among trained pigs (n =143) as described in Figure 1. While a few pigs contacted the rope in a single contact event, the majority cycled away and back to the rope over the observation period.



observed pigs contacted the rope in the first 10 minutes, 59 pigs (65.6%) in 20 minutes, and 63 pigs (70.0%) in 30 minutes; 27 pigs (30.0%) never contacted the rope. Cumulatively, it took 64 minutes for the untrained pigs to reach a 70% participation level.

Discussion

The use of oral-fluid-based surveillance facilitates health monitoring while providing a positive image for pork producers. That is, the use of oral fluids shows that we are dedicated to improving animal welfare by providing a stress-free procedure to monitor populations of pigs for a variety of infectious agents. Rope-based oral-fluid collection from pigs is possible because it is compatible with normal behavior. That is, pigs are naturally curious and explore their environment by biting, chewing, and tasting.[10,11] Age, but not gender, has been shown to affect the level of interaction, with 13-week-old pigs exhibiting more activity than 5-week-old pigs, which in turn were more active than 3-week-old pigs.[12] Pigs have a particular preference for objects that are chewable, flexible, and destructible,[12-15] eg, rope.

In this study, analysis of the data led to the formulation of two sampling recommendations: one for pigs with prior exposure to oral-fluid sampling (trained pigs) and one for those with no prior experience (untrained pigs). In trained pigs, a ≥ 20-minute oral-fluid sampling period is recommended in order to assure the participation of approximately 70% of the pigs in pens of 25 to 30 pigs. In untrained pigs, a 60-minute oral-fluid sampling is recommended to achieve a similar level of participation. The data in this study suggest that one collection experience is sufficient to train pigs. Future research is required to develop data-driven sampling recommendations for pens of different design and size.

Implications

- Oral-fluid collection can easily be added to a normal walk-through or integrated into a routine surveillance program.
- Collect samples using 100% cotton rope; hang the end of the rope at pig shoulder level.
- Allow a minimum of 20 minutes for trained pigs to interact with the rope, 60 minutes if pigs have not had prior experience with oral-fluid collection.

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Figure 4: Percent of "untrained pigs" (n = 90) interacting with the rope over the first observation period. Observation periods described in Figure 1. Untrained pigs had no previous experience with oral-fluid collection, in contrast to the trained pigs (described in Figure 1).

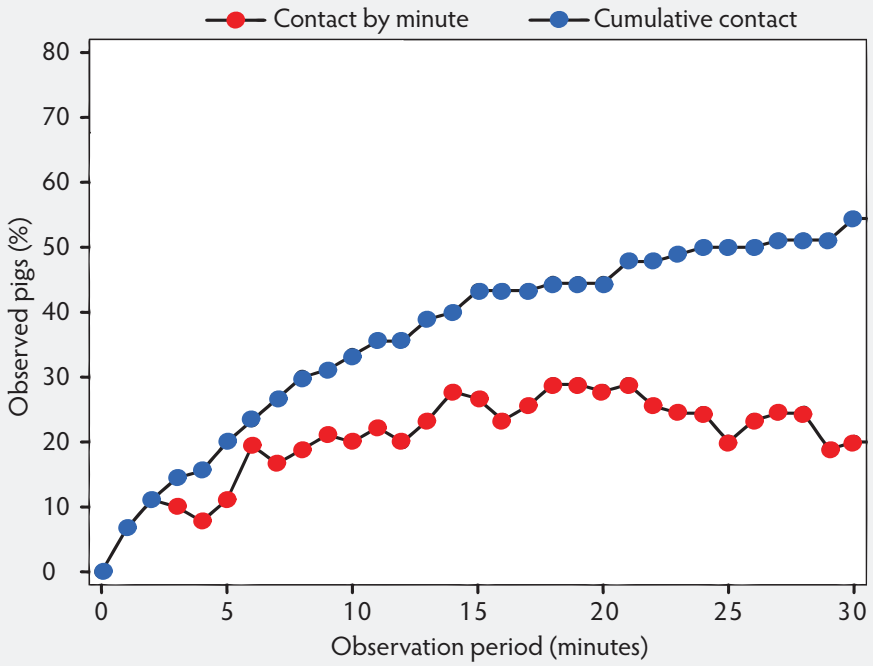
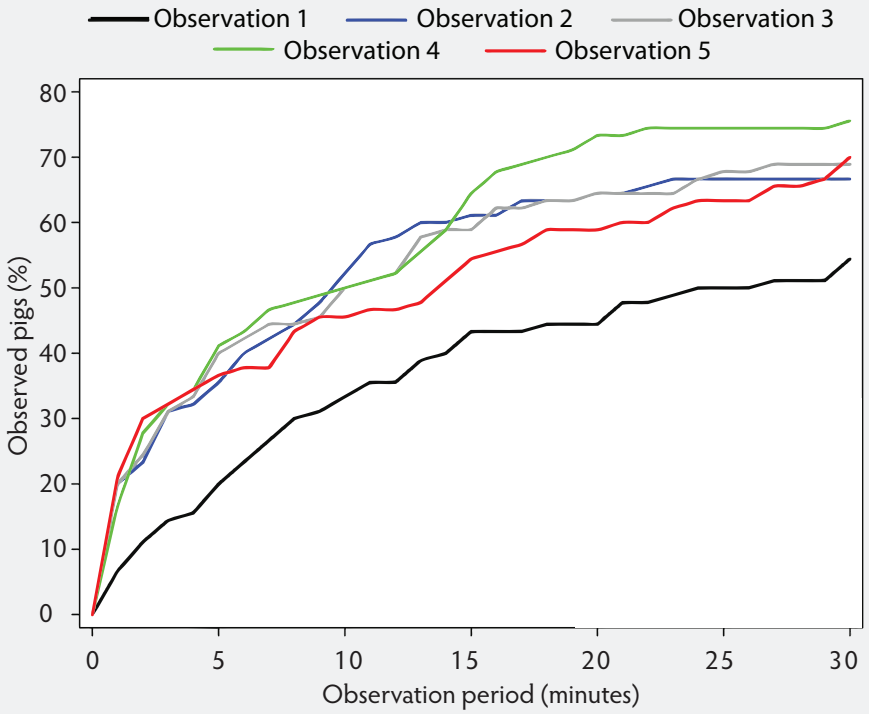


Figure 5. Percent of untrained pigs (n = 90; described in Figure 4) interacting with the rope (cumulative contact) over five sequential observation periods (described in Figure 1).



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